[SEQ CHAPTER \h \r 1]
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM:

To: Eric Bohnenblust, Ph.D.
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From: Chris A. Wozniak, Ph.D.
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Through: Shannon Borges, Senior Scientist
Risk Assessment Branch
Biopesticides and Pollution Prevention Division

Date: [DATE \@ "M/d/yyyy"]

Evaluation of the Response to the 10-Day Deficiency Letter for the Environmental Risk Assessment for the New Product OX513A (EPA File Symbol: 93167-EUP-3) Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV; Designated as the New Active Ingredient) Protein, the Modified *Discosoma* spp. DsRed2 Protein (Designated as a New Inert Ingredient), and the Genetic Material (Vector pOX513) Necessary for Their Production in OX513A *Aedes aegypti*; Data and Information Were Provided in Support of a FIFRA Section 5 Experimental Use Permit.

THIS DOCUMENT DOES NOT CONTAIN CONFIDENTIAL BUSINESS INFORMATION

DP barcode: 445454
Decision no.: 537030
Submission no: 1013127
Action code: B620-New a.i.; EUP. Non-food use including crop destruct
Product Name: OX513A
EPA Reg. No or File Symbol: 93167-EUP-R
Formulation Type: Male mosquitoes
Ingredients statement from the label with PC codes included: a.i. 8.25 %
PC: 006002 (tTAV), 829083 (DsRed2)
MRID Number(s): 50326401, 50443507, 50443508, 50443509, 50443510, 50443511, 50443512, 50443513, 50443514, 50443515, 50443516, 50443519

A. Data Deficiencies: Yes/No

The specific data requested by the Agency are:

1. Specific data needed are: Explanation regarding the methods used to quantify tTAV and DsRed2 proteins is still needed; current data presentation is less than clear for Western blots in that a failure to detect tTAV and DsRed2 in larvae and adult OX513A extracts leaves quantitation nebulous. The Agency is left to conclude that levels of these two proteins are low, but at unknown levels. See Product Characterization / Human Health Response to the 10-Day Deficiency Letter Memo for further details. The response to the 10-day letter did not provide sufficient information for EPA to complete the assessment.

B. Label deficiencies: Yes/No

Some explanation as to how the applicant arrived at 8.25% a.i. for the tTAV protein is warranted given the lack of quantitation demonstrated.

ACTION REQUESTED:

Oxitec Ltd., (Oxitec) requests an Experimental Use Permit (EUP) under FIFRA section 5 for a new end-use product containing the new active ingredient tetracycline-repressible transactivator protein variant (tTAV) protein, the new inert ingredient DsRed2 modified protein, and the genetic material (vector pOX513) necessary for their production in the Yellow Fever Mosquito, *Aedes aegypti* OX513A for the suppression of *Ae. aegypti* populations.

This screening memo is intended to assess the integrity of the data presented in the listed MRIDs in support of the EUP for OX513A's limited release into the environment. Data and information relevant to the human health and product characterization assessment are detailed in a separate screening memo.

I. Conclusions:

Much of the environmental risk assessment will be premised upon minimal exposure of non-target organisms to the a.i., tTAV protein. Western blot procedures intended to quantify the tTAV and DsRed2 (inert) proteins have significant issues which compromise the integrity of the results (See Product Chemistry / Human Health screening memo). The inability to detect either tTAV or DSRed2 proteins with the appropriate polyclonal antibodies and render some quantitative measure or estimate of concentration hampers the ability to derive an Estimated Environmental Concentration for the active ingredient. Reaching an accurate conclusion on the presence or absence (failure to detect) of tTAV in larvae and adult *Aedes aegypti* is difficult at best given the results presented; spiking the extracts with known quantities of protein may be helpful to demonstrate the lack of proteolytic activity in the extracts.

Other studies appear to contain all necessary components to provide for appropriate review and determinations relative to the environmental risk assessment.

SUMMARY OF STUDIES REVIEWED FOR TECHNICAL SCREEN

MRID No.	TITLE	FINDING	COMMENT
50326401	Fish Acute Oral Toxicity, Freshwater	Sufficient for review	
50443507	Freshwater Invertebrate LC_{50}	Sufficient for review	
50443508	Avian Acute Oral Toxicity	Sufficient for review	Rationale for waiver
50443509	Avian Dietary Toxicity	Sufficient for review	Rationale for waiver
50443510	Terrestrial Plant Toxicity (Seedling Emergence)	Sufficient for review	Rationale for waiver
50443511	Terrestrial Plant Toxicity (Vegetative Vigor)	Sufficient for review	Rationale for waiver
50443512	Nontarget Insect Testing	Sufficient for review	Rationale for waiver
50443513	Environmental Assessment (FDA)	Sufficient for review	Supplemental materials for ERA
50443514	Endangered Species Assessment	Sufficient for review	Supplemental materials for ERA
50443515	Effects of Environmental Exposure to Tetracycline	Sufficient for review	
50443516	Bioinformatics analysis for predicting protein digestibility and environmental degradation of OX513A tTAV and DsRed2 proteins by proteases, and likelihood of these proteins crossing a cell membrane	Sufficient for review	
50443519	On Breeding Phenotype of Surviving OX513A Reared in the Absence of Tetracycline	Sufficient for review	